A STUDY ON INSULIN RESISTANCE IN POLYCYSTIC OVARY SYNDROME

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CERTIFICATE

This is to certify that this dissertation in **"A STUDY ON INSULIN RESISTANCE IN POLYCYSTIC OVARY SYNDROME"** is a work done by **Dr.R. SHANTHI,** under my guidance during the period 2004 - 2007. This has been submitted in partial fulfillment of the award of M.D. Degree in Biochemistry, (Branch - XIII) by the Tamil Nadu Dr.M.G.R. Medical University, Chennai - 600 032.

THE DEAN
Madras Medical College &
Govt. General Hospital,
Chennai - 600 003.

Date :

Station :

Prof.A.MANAMALLI, M.D.,
Director & Professor
Department of Biochemistry
Madras Medical College,
Chennai - 600 003.

Date :

Station :

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CONTENTS

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most frequent androgen disorder in women. A prevalence of 5% - 10% of PCOS was found in women of reproductive age. These patients have a tendency to abdominal fat accumulation and over weight which is partly associated with over production of ovarian androgens. The increased insulin levels has been attributed to insulin resistance which has been suggested as the principle underlying disorder of PCOS. Insulin resistance is defined as a decreased biological response to normal levels of circulating insulin and is found in obese non diabetic people, type 2 diabetes and those women with polycystic ovarian syndrome. The percentage of PCOS women with insulin resistance was found to vary in the results given by different workers in this field. As per their work in western population it varied from 60% to 80%. The varying high percentage obtained by previous workers created the interest and hence this work on insulin resistance in PCOS has been selected with the view of determining the percentage of PCOS with insulin resistance in our population.

REVIEW OF LITERATURE

Polycystic ovary syndrome is a common condition characterised by menstrual abnormalities and clinical or biochemical features of hyper androgenism[1]. Interest in polycystic ovary syndrome has increased recently with the realization that this syndrome involves multisystem effects which includes organs other than the reproductive system. Initially called the stein - leventhal syndrome in 1930s, PCOS is now recognised to be a metabolic syndrome which may include hyperinsulinemia, hyperlipidemia, diabetes mellitus and possibly cardiac disease, and also the more conventionally recognised increase in androgen levels, cosmetic problems, anovulation, infertility, endometrial cancer and obesity[2,3].

INCIDENCE

The percentage of PCOS in women of child bearing age was found to be 5 - 10% as per Robert J. Norman et al., who have based their study on the diagnostic criteria of US National institute of Health. Moreover as per their study the percentage of PCOS increased to 30% in women who are infertile[4,5,13].

PCOS is a life long condition and its effects are found to manifest at all ages. Depending on the age group the manifestations are premature puberty during childhood, hirsuitism and menstrual abnormalities during teenage, infertility and glucose intolerance during early adulthood and middle life and diabetes mellitus and cardiovascular disease in later life. The causes for the above manifestations are tabulated in Chart No.1[1].

CHART NO.1

MANIFESTATIONS OF PCOS AT DIFFERENT AGES[1]

Inutero	**Peripuberty**	**Adolescence & adulthood**	**Ageing**
Small baby syndrome	**Exaggerated adrenarche**	**PCOS**	**Metabolic syndrome**
Intrauterine growth retardation	Increased levels of * Adrenal androgens * Insulin - Functional ovarian hyperandrogenism	- Anovulation - Hyperandrogenism - Polycystic ovaries - Obesity (50%)	- Diabetes - Hypertension - Dyslipidaemia - Increased plasminogen activator inhibitor-1
Leads to long term health defects	Leads to precocious puberty	Leads to reproductive disorder	Leads to metabolic effects

PATHOGENESIS

Pathogenesis of PCOS is poorly understood, but the primary defect may be insulin resistance, androgen excess and abnormal gonadotropin dynamics[6]. Recent evidence suggests that the principal underlying disorder is one of insulin resistance, with resulting hyperinsulinemia stimulating excess ovarian androgen production[7,8]. There is a failure to ovulate, the follicles becoming cystic and shows hyperplasia of theca interna. It is supposed that the hyperthecosis is related to an overproduction of androgens which reduces granulosa cell proliferation and maturation as well as stimulating fibrosis of surrounding stroma and capsule. Though there are no specific histological features which charaterize the ovary in PCOS, follicular as well as theca lutein cyst may be present. Moreover ripening follicles and occasionally an active corpus luteum can be found. This shows that ovulation may occur intermittently despite thick capsule.

Irrespective of microscopic appearances, it is clearly seen that there is no defect in the hypothalamic - pituitary - ovarian axis. However, normal function is masked by inhibition of ovarian follicular development and in appropriate feedback to the pituitary. The high estrogen production is largely due to conversion of androgens to estrogen in the ovary and peripherally. This causes an increase in Luteinizing hormone (LH) and a decrease in follicular stimualting hormone (FSH). A vicious circle is established that the increase in LH induces thecal hyperplasia and increased androgen synthesis in ovary.

High levels of androgen result in the peripheral production of estrogen and reduction in sex hormone - binding globulin. This leads to an increased level of free androgens to produce hirsuitism and to be converted to estrogen[9].

The elevated androgens contribute to the morphologic effect within the ovary preventing normal follicular development and inducing premature atresia. In another aspect of vicious cycle, the local androgen block is a major obstacle that maintains the steady state of persistent anovulation. When ovarian androgen concentrations are high they can be converted to 5α - reduced metabolites which inhibit aromatase activity and estrogen production[10].

Following wedge resection of ovaries the androgen level is reduced and ovulatory cycles return. This shows that the intraovarian androgen effect is a principal factor in preventing normal cycling[11]. In this manner the classic picture of polycystic ovary is attained, displaying numerous follicles in early stages of development and atresia and dense stromal tissue. Thus the polycystic ovary is the result of "vicious cycle", which can be initiated at any one of many entry points. Altered function at any point in the cycle leads to polycystic ovary.

MORPHOLOGY

The polycystic ovary is usually enlarged and characterised by a smooth pearly white capsule. The surface area is doubled and this gives an increase of 2.8 times the average volume. Same number of primordial follicles are present, but the number of growing and atretic follicles is doubled. Each ovary may contain 20 - 100 cystic follicles. Thickness of tunica is increased by 50%. There is increase in cortical stromal thickness. On cut section it shows cystic follicles and hyperplasia of theca interna[12]. The gross appearance of polycystic ovary is shown in Fig.1 and 2.

CLINICAL FEATURES

In many women the symptoms are easily recognizable, but ethnicity influences the extent of symptoms. The national institute of child health and development held a consensus meeting to develop the following diagnostic criteria for PCOS.

1. Clinical or biochemical evidence of hyperandrogenism.

2. Menstrual irregularity due to oligo ovulation or anovulation.

3. Exclusion of other known disorders such as congenital adrenal hyperplasia or hyperprolactinemia.

Hyperandrogenism : The wide spectrum of manifestations ranges from mild acne and increased terminal (coarse) hair growth on midline structures (Face, Neck, Abdomen) to android changes in body habitus with waist-to-hip ratios of more than one. In addition non hirsute women with anovulation may have laboratory evidence of hyperandrogenism.

Frank or rapid virilization including clitoromegaly, vocalcord thickening or male pattern baldness are rare in PCOS but when present suggests another cause of hyperandrogenesim like adrenal disorders or androgen producing tumors.

Menstrual irregularity : Manifest as oligo ovulation or anovulation and present as oligomenorrhoea or amenorrhoea. Among women with more regular menses many have variable degrees of ovulatory dysfunction. Often the normal menstrual flow of 3 - 5 days / 28 - 35 days occurs at first on

attaining menarche but becomes less frequent after a period of 2 years occurring once during 45 to 365 days[14]. Because the estrogen from ovarian and adipose tissue stimulates proliferation of endometrium that is not stabilized by post ovulatory progesterone, bleeding can be unpredictable, heavy, and prolonged.

SUGGESTED DIAGNOSTIC CRITERIA FOR PCOS[15]

1. Clinical Features

- Amenorrhoea, oligomenorrhoea or dysfunctional uterine bleeding
- Anovulatory infertility
- Hirsutism and / or acne
- Central obesity

2. Endocrine Abnormalities on Laboratory Tests

- Elevated androgen (testosterone levels)
- Elevated LH concentration with normal to mildly elevated FSH level
- Insulin resistance with hyperinsulinemia.

3. Radiologic abnormalities on USG examination

- Multiple (nine or more) subcortical follicular cysts
- Increased ovarian stromal density and / or volume

4. Exclusion of other etiologies

- Prolactinoma
- Virilizing tumours of adrenal or ovarian tumors
- Congenital adrenal hyperplasia
- Cushing's syndrome

The diagnosis is based on the presence of some or all of the common clinical features and is confirmed by the presence of biochemical or radiological evidence of endocrine abnormality and exclusion of other etiologies[15].

According to Marilyn R. Richardson the evaluation should follow these principles.

1. Detect hyperandrogenism
2. Exclude other causes of hyper androgenesim
3. Exclude other etiologies of amenorrhoea and to confirm by Laboratory investigations which are tabulated in Chart - 2[14].

CHART - 2
LABORATORY INVESTIGATION OF PCOS[14]

Test	Normal value	Purpose
b-hCG	< 5 mlU per mL (<5 lU per L)	Exclude pregnancy
TSH	0.5 to 4.5 µU per mL (0.5 to 4.5 mU per L)	Exclude thyroid dysfunction
Prolactin	< 20 ng per mL (<20 µg per L)	Exclude hyperprolactinemia
Testosterone (total)	< 20 ng per mL (<0.7nmol per L)	Exclude androgen - secreting neoplasm
Testosterone (free)	20 to 30 years - 0.06 to 2.57 pg per mL (0.20 to 8.90 pmol per L) 40 to 59 years - 0.4 to 2.03 pg per mL (1.40 to 7.00 pmol per L)	Establish diagnosis or monitor therapy
DHEAS	600 to 3,400 ng per mL (1.6 to 9.2 µmol per L)	Exclude androgen - secreting neoplasm
Androstenedione	0.4 to 2.7 ng per mL (1.4 to 9.4 nmol per L)	Establish diagnosis
17a-hydroxyprogesterone	Follicular phase <2 µg per L (6.1 nmol per L)	Establish - Non classical Adrenal hyperplasia (NCAH)
Fasting insulin	< 20 µU per mL (<144 pmol per L)	Exclude hyperinsulinemia
Fasting glucose	65 to 119 mg per dL (3.6 to 6.6 mmol per L)	Exclude type 2 diabetes or glucose intolerance
Fasting glucose : insulin ratio	> = 4.5	Exclude insulin resistance
Cholesterol (total)	150 to 200 mg per dL (1.5 to 2 g per L)	Monitor lifestyle changes
HDL cholesterol	35 to 85 mg per dL (0.9 to 2.2 mmol per L)	Monitor lifestyle changes
LDL cholesterol	80 to 130 mg per dL (2.1 to 3.4 mmol per L)	Monitor lifestyle changes
Endometrial biopsy	Negative for hyperplasia / malignancy	Exclude malignancy or hyperplasia

INSULIN RESISTANCE

Insulin resistance refers to the clinical condition in which the biologic response to insulin is diminished to below normal levels[16]. The decreased response can be the result of 2 states namely of either decreased sensitivity to insulin or decreased responsiveness to insulin or a combination of both.

Decreased Sensitivity to insulin : There is a rightward shift in dose response to the hormone but the maximal response remains normal.

Decreased Responsiveness to insulin : The dose response is normal but the maximal response is decreased.

The two states of insulin resistance are depicted as a graph in Fig.No.3[17].

From molecular perspective insulin resistance can occur at multiple levels which can be prereceptor, receptor or post receptor levels.

LEVELS OF INSULIN RESISTANCE

1. Pre receptor insulin resistance

This is rare today but formerly shown by patients with high levels of circulating antibodies to insulin. These antibodies blocked the binding of ligand to its receptor[18].

2. Insulin resistance at the level of receptor

This may be due to genetic alterations in :

a. Receptor expression or structure (The normal structure is elaborated in Fig.No.4).

b. Secondary changes in receptor activity due to serine phosphorylation.

c. Down regulation of receptor concentration.

3. Insulin resistance at post receptor level

This can occur any where on one of the common or branches pathways of insulin signaling.

In most common states of insulin resistance the defects are at multiple levels. For example in type 2 diabetes insulin resistance is associated with a decrease of the following :

- receptor concentration
- receptor kinase activity
- concentration and phosphorylation of insulin receptor substrates (IRS-I and IRS-2)
- phosphatidyl ionositol (PI)-3 kinase activity
- glucose transport translocation and
- defects in activity of intracellular enzymes[19,20].

Insulin resistance can either be due to genetic causes or acquired causes.

Insulin resistance due to genetic defects in insulin receptor expression or sequences is relatively rare. This occurs in most severe forms of insulin resistance. In humans they may present as different disease syndromes like

Leprechaunism, Rabson Mendenhall Syndrome and type A syndrome of insulin resistance. Though there is some correlation between the severity of the genetic defect in receptor function and the severity of the clinical presentation, the correlation is relatively weak, indicating that other genetic or acquired factors can modify the insulin resistant state significantly[21].

ACQUIRED FORM OF INSULIN RESISTANCE

This occurs due to multiple mechanisms which are illustrated in Fig.5 and are enumerated below :

1. Insulin receptor down regulation[22,23]

In this condition there is mild hyper insulinemia due to tissue resistance, which results in internalization and increase in degradation of insulin receptors. This occurs in obesity and type 2 diabetes.

2. Down regulation in insulin receptor substrates[24,25,27]

Hyperinsulinemia can lead to down regulation of IRS producing greater decrease in insulin signaling. In humans, the levels of insulin receptor and insulin receptor substrate - I in some tissues can be reduced by more than 50% in some of these insulin resistant states.

3. Increase serine phosporylation of receptor and its substrates[26,28,29]

This leads to decreased kinase activity of the receptor and decreased

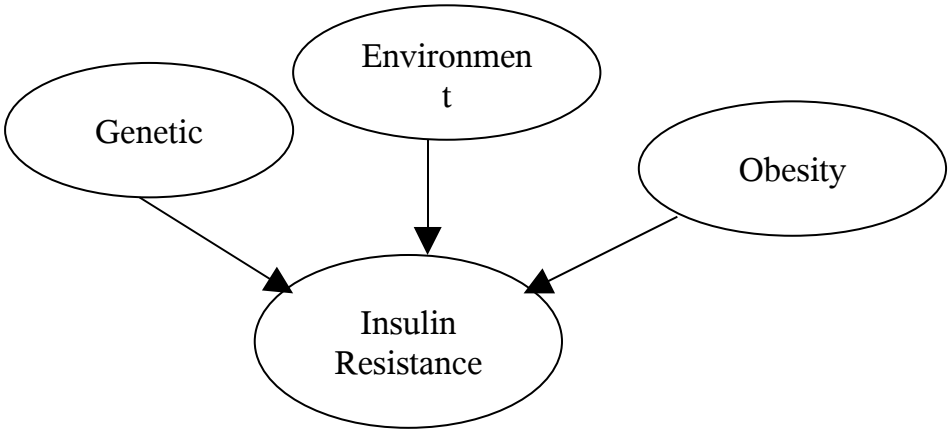
tyrosine phosphorylation of the receptor substrates by protein tyrosine phosphatases (PTPase). Serine phosphorylation is caused by several serine kinases, like protein kinase C (PKC), proteinkinase B (PKB), mitogen activated protein kinases (MAP).

The upstream stimulators of these serine kinases are also multiple. For example in obesity and type 2 diabetes there is increased level of circulating free fatty acids while in obesity tumor necrosis factor α (TNF α), leptin and other peptides are made and released from adipose tissue. Another class of proteins act as inhibitors of insulin signaling. They are suppressor of cytokines signalling (SOCS) proteins. These proteins interact with phosphorylated receptor and block insulin action[30,31,32]. These play a role in insulin resistance in stress induced states like bacterial infection and type II diabetes.

Clinical Manifestations of genetic conditions of insulin resistance are hyperinsulinemia, dyslipidemia, hyper-tension and impaired glucose tolerance or insulin resistant diabetes[33]. Acanthosis nigricans is a characteristic skin lesion in insulin resistance consisting of velvety and papillomatous pigmented hyperkeratosis of flexuses and neck[34]. In women extreme insulin resistance is also associated with hyperandrogenism, hirsuitism, menstrual abnormalities and polycystic ovary disease.

Though several factors contribute to insulin resistance, genetic inheritance, obesity and environment which include a variety of factors like hormones, increased nutrient availability and age play a very important role in

its pathogenesis, which is illustrated below :



GENETIC

Defects in both insulin action and insulin secretion are present in type 2 diabetes and both are believed to be genetically predetermined. A strong genetic basis for insulin resistance is suggested by the high prevalence in certain populations. To date several rare mutations or genes associated with insulin action have been linked to extreme insulin resistance. Though these are all rare conditions, their study has been helpful in elucidating the general pathophysiology of insulin resistance. Severe insulin resistance has been reported in conjunction with more than 100 naturally occurring mutations in the insulin receptor gene, as well as in the complete absence of insulin receptors[35,36,37]. The varying severity and diversity of their phenotypes have resulted in the description of several clinical syndromes like leprechaunism, Rabson - Mendenhall syndrome, and Type A insulin resistance.

OBESITY

The quantity of body fat varies widely in mammals ranging from 2% to 50% of body mass. This large variation in fat mass is apparently determined both by an individual's genetic background and by environmental factors, including diet and physical activity. Excess body fat, or obesity, is an important factor in the pathogenesis of insulin resistance, and substantially increase the risk of type 2 diabetes[38,39,40]. While adipose tissue influences whole-body insulin action both through release of free fatty acids and by secretion of adipose derived proteins. They include both pro-inflammatory peptides like tumor necrosis factor α (TNF α), interleukin - 6 (IL-6), transforming growth factor β, C - reactive protein, monocyte chemotactic protein - 1 (MCP-1) and newly identified hormones like adiponectin, leptin and resistin[82,83]. Decreased circulating levels of adiponectin and defective leptin action accompany obesity and insulin resistance. Adipose - derived proinflammatory moleceules are believed to induce systemic insulin resistance. In obesity the levels of these circulating adipokines are increased[17]. TNF - α leads to insulin resistance by increasing serine phosphorylation of IRS-I and decreasing insulin - receptor kinase activity[41].

ENVIRONMENT

This refers to variety of factors including hormones, increased nutrient availability and age. Hyperinsulinemia is the classic indicator of insulin resistance and may itself contribute to insulin resistance in type 2 diabetes and

obesity. Growth hormone acts at several levels to block insulin action contributing to insulin resistance including the inhibition of phosphorylation of the insulin receptor and IRS-I in response to insulin administration[42]. Increased nutrient intake like increased glucose and free fatty acids also causes insulin resistance. Final environment factor is advancing age which also contribute to insulin resistance. The features of aging like increased fat mass particularly increased viseral obesity, increased circulating levels of inflammatory proteins, and increased cellular accumulation of triglycerides contribute to insulin resistance[43,44].

Insulin resistance occurs both in physiologic states and disease conditions which are enumerated below :

Physiologic conditions

- Puberty
- Pregnancy

Disease states

- Type 2 diabetes
- Obesity
- Hypertension
- Polycystic ovarian disease
- Variety of genetic syndromes which are classified as genetic defects in insulin receptor, immunologic mechanisms and disorders of unknown etiology and are tabulated in Chart No.3.

Insulin resistance is also present in many states of stress, in association with infection and secondary to treatment with variety of drugs, particularly glucocorticoids[19].

CHART NO. 3

GENETIC SYNDROMES WITH SEVERE INSULIN RESISTANCE[16]

Genetic defects in insulin receptor

- Type A syndrome
- Rabson mendenhall syndrome
- Leprechaunism

Immunologic, autoimmune

- Antibodies to insulin receptor

  Type B syndrome

  Ataxia - telangiectasia
- Antibodies to insulin

Disorders of unknown etiology : Lipotrophic diabetes

Congenital-Dominant inheritance (Kobberling - Dunningan Syndrome)

- Autosomal recessive inheritance (Seip Syndrome)

Acquired - Generalised (Lawrence Syndrome)

- Partial lipoatrophy

INVESTIGATIONS FOR EVALUATION OF INSULIN RESISTANCE

1. Fasting Insulin Levels

Fasting serum insulin level greater than the upperlimit of normal for the assay used is considered evidence of insulin resistance. With normally functioning pancreatic β cells, fasting and peak plasma insulin concentration are directly related to the degree of peripheral insulin resistance as elaborated below.

1. Fasting plasma insulin 2 to 25 μu/ml is the reference range for insulin.

2. Fasting plasma insulin 25 to 50 μu/ml - mild to moderate insulin resistance and rarely exceeds 50 μu/ml.

3. Fasting plasma insulin close to normal or markedly elevated 200 - 400 μu/ml in extreme insulin resistance[16].

2. Oral Glucose Tolerance testing (OGTT)

During this test a fasted patient takes 75 gm oral dose of glucose and blood glucose levels are measured over the following 2 hours. This may be used to diagnose diabetes mellitus. This can be normal or mildly abnormal in simple insulin resistance.

3. Hyperinsulinemic euglycemic clamp

The gold standard for investigating and quantifying insulin resistance is the "hyperinsulinemic euglycemic clamp". It is so called because it measures the amount of glucose necessary to compensate for an increased insulin level without causing hypoglycemia. With this technique insulin is infused in gradually increasing amounts, along with administration of glucose to prevent fall in plasma glucose. The steady - rate of peripheral glucose utilization at several insulin levels is measured as milligrams of glucose used per kg of body weight per minute. The values provide an estimation of the sensitivity of peripheral tissues to exogenous insulin administration. This is performed at specialized medical centres and not required for clinical evaluation[16].

Other atlernative methods of evaluation of insulin resistance are glucose to insulin ratio and homeostatic model assessment[45].

A fasting glucose to insulin ratio is a useful measure of insulin sensitivity and according to studies it provided the best single fasting measure of insulin action and was comparable with glucose stimulated parameters. A fasting G/I ratio <4.5 has significant correlation with insulin resistance and has been studied for use as a screening test in PCOS[14].

The homeostatic model assessment value for insulin resistance (HOMA IR) is calculated as follows :

HOMA IR = [(Glucose in mg / dl x 0.05551) x insulin in micro units / ml] / 22.5.

Glucose and insulin are fasting plasma values. Glucose in the formula is mmol/l (mg/dl x 0.05551) and insulin is in micro units / ml[46]. HOMA IR levels >3.8 is indicative of insulin resistance as per Dr.Elizabeth G. Nabel[89].

INSULIN RESISTANCE IN PCOS

The association between a disorder of carbohydrate metabolism and androgen excess was first described in 1921 by Archard and Thiers[80]. and was called the "diabetes of bearded women". Since then the association between PCOS and insulin resistance or impaired glucose tolerance has been well recognised[47]. Insulin resistance accompanied by compensatory hyperinsulinemia is recognised as the most significant metabolic aberration in polycystic ovary syndrome[46]. It has become increasingly evident that insulin resistance plays a significant role both as a cause and result of the syndrome[48].

The resistance to the action of insulin in PCOS primarily refers to impaired action of this hormone on glucose transport and antilipolysis on adipocytes in the presence of normal insulin binding[49-51]. In turn the compensatory hyperinsulinemia leads to an exaggerated effect of insulin in other less traditionally responsive tissues including stimulated androgen secretion by the ovarian theca cells[52,53]. The insulin resistance which underlies the androgenic features of the disorder is highlighted by the observation that the administration of insulin sensitizing agents is associated with an improvement in clinical, endocrinologic and metabolic features in many patients with PCOS [54-57].

The state of insulin resistance in PCOS is though not well understood it can be due to :

1. Peripheral target tissue resistance to insulin which can be the result of autoantibodies to insulin receptors leading to decraese in insulin binding; Post receptors defects[59].

2. Decreased hepatic clearance of insulin due to decreased hepatic extraction[60].

Current research has focused on identifying a genetic predisposition for insulin resistance in this syndrome[48].

In order to understand the molecular defect underlying insulin resistance in PCOS, Dunaif and co-workers[58,47] studied the differences between skin fibroblasts from women with and without PCOS with respect to insulin dependent signal transduction. The fibroblasts of women with PCOS showed no change in insulin binding or receptor affinity[59]. In fibroblasts the mitogenic pathway of insulin action is intact and this is consistent with a selective defect in insulin signaling. While in PCOS the skeletal muscle is resistant to insulin in vivo, cultured muscle cells have normal insulin sensitivity, consistent with a major role of extrinsic factors in producing insulin resistance in the tissue[58]. However a post receptor defect was observed. This defect is characterized by increased basal insulin receptor serine phosphorylation and there is decreased in insulin dependent tyrosine phosphorylation of the insulin receptor. These abnormal patterns of phosphorylation of specific residues of the insulin receptor might represent a molecular mechanism responsible for the insulin

resistance, anovulation and androgen excess in PCOS.

The androgen excess and insulin resistance are often associated with acanthosis nigricans which is a grey brown velvety discolouration around skin of neck, groin and axillae and is a marker for insulin resistance. The presence of acanthosis nigricans in hyperandrogenic women is dependent on the presence and severity of insulin resistance and hyperinsulinemia. But it is not specific for insulin resistance as it can be seen in the absence of insulin resistance or androgen excess.

Insulin resistance is frequently observed in both non obese and obese women with PCOS[59]. In studies that were performed to determine whether insulin resistance occurred in patients with PCOS in the absence of obesity and acanthosis nigricans, significant degree of insulin resistance was found to exist in non obese patients with PCOS in whom the insulin resistance was significantly related to LH and free testosterone concentration[60]. Insulin resistance observed in the absence of overweight can be secondary to a defect in the insulin transduction pathway mainly by a defect in receptor phosphorylation. This enhances hyperandrogenism by increasing ovarian androgen production[61]. More severe degrees of insulin resistance or impaired glucose tolerance are more common in obese women with PCOS[47]. In such patients obesity perse is the cause of insulin resistance[60]. In coexistance of obesity and insulin resistance in PCOS, there is excess cardivoascular risk which is contributed by obesity. Most of these obese patients exhibit higher

body mass index, androgen level and TGL levels and decrease in HDL - c levels. Insulin resistance was the main determinant of HDL - c and TGL levels in PCOS[62]. Hence measures to decrease insulin resistance must be considered earlier to minimise the potential risk of developing diabetes and coronary artery disease at later ages of life in these patients.

The clinical presentation of insulin resistance depends on the ability of pancreas to compensate for target tissue resistance. When compensation is effective it leads to hyperinsulinemia[59]. Evidence for hyperinsulinemia contributing to the androgen excess by stimulating the theca cells is strong[63]. The existence of stimulatory action of insulin on LH - secretion will help to explain the elevated LH concentration in this syndrome. Another action of insulin is to decrease the sex hormone binding globulin synthesis in liver thereby leading to an increase in free androgen concentration[64]. Further hyperinsulinemia potentiates adrenal androgen secretion by ACTH stimulation[65]. It contributes to increased risk of cardiovascular disease by means of direct atherogenic action and indirectly affecting of lipoprotein (LP) profile[12]. Hyperinsulinemia may be important factor in the infertility associated with PCOS[65]. Hyperinsulinemia therefore is a key component of PCOS.

[12]There are studies indicating that androgens can induce hyperinsulinemia. However most of the evidence supports hyperinsulinemia as a primary factor, especially the experiments in which turning off the ovary with a Gonadotropin releasing hormone (GnRH) agonist does not change the

hyperinsulinemia or insulin resistance. This indicates that disordered insulin action precedes the increase in androgens. Indeed there are many reasons to believe that hyperinsulinism causes hyperandrogenism which are enumerated below.

1. Administration of insulin to women with polycytic ovaries increases circulating androgen levels[66].

2. Administration of glucose to hyperandrogenic women increases the circulating levels of both insulin and androgens[67].

3. Weight loss decreases the levels of both insulin and androgens and increases the levels of insulin like growth factor binding protein[68].

4. In vitro insulin stimulates theca cell androgen production[69,70].

5. The experimental reduction of insulin levels in women reduces androgen levels in women with polycystic ovaries but not in normal women[71-73].

6. After normalization of androgens with GnRH agonist treatment the hyperinsulinemia response to glucose tolerance testing remains abnormal in obese women with polycystic ovaries[74-75].

7. Correlation of hyperandrogenism with oral contraceptive treatment, surgical wedge resection or laproscopic ovarian

cautery does not restore insulin resistance and abnormal lipid and lipoprotein levels to normal[76].

Measures of insulin resistance in PCOS included fasting serum insulin, glucose to insulin ratio and homeostasis model assessment (HOMA)[45]. These women also exhibit elevated levels of C reactive protein and endothelium dysfunction which are directly correlated with insulin resistance[77].

A prospective case control study was done to determine the prevalence of insulin resistance in patients with PCOS. Compared with PCOS patients without insulin resistance, patients with insulin resistance were more obese and had high beta cell function. The prevalence of insulin resistance was 64% according to HOMA - IR measurement[78]. Although insulin resistance is a common abnormality in PCOS it doesn't seem to be an universal feature[78]. In PCOS insulin resistance was diagnosed in 65.4% using glucose insulin ratios. In obese women (body mass index >28 in 48% of group). IR was present in 76.7% as measured by glucose / insulin ratios but significantly higher (95.3%) using values of either HOMA or QUICK I (Quantitative insulin sensitivity check index). According to Negro RS et al., approximately 50% to 70% of all women with polycystic ovary syndrome have some degree of insulin resistance[80]. Moreover different studies show that insulin resistance is also seen in parents and sisters of women with PCOS[81]. Although uncertainty exist early detection and treatment of insulin resistance in this population could ultimately reduce the incidence or severity of diabetesmellitus, dysplipidemia, hypertension and cardiovascular disease[80].

AIM OF THE STUDY

On having reviewed about PCOS, insulin resistance and the prevalence of the latter in PCOS which leads to hyperinsulinemia and hyperandrogenism the present study was taken up with the view of finding out how many subjects with PCOS had insulin resistance. To determine this the following work was undertaken in apparently normal subjects and those with PCOS.

1. Determination of the level of fasting plasma glucose and fasting plasma insulin.

2. Calculation of plasma glucose insulin ratio and homeostasis model assessment - IR.

3. Determination of the percentage of PCOS women with insulin resistance.

4. Determination of lipid parameters as hyperlipidemia is one the complication of PCOS.

5. Correlation of plasma insulin to plasma glucose and lipid parameters.

MATERIALS AND METHODS

The study was done during the period Jan-2006 to April 2006. It was carried out in 2 groups namely control and PCOS subjects in the reproductive age group (20 - 40 yrs).

Control Group

The group comprised of 30 apparently healthy female subjects with regular menstrual cycle. They were selected from the staff of biochemistry department Madras Medical College, Chennai and females who accompanied the patients attending endocrinology outpatient department (OPD) at the Institute of Obstetrics and Gynaecology, Egmore, Chennai.

Test Group

This group comprised females with PCOS who had history of menstrual irregularities / infertility and were attending the endocrinology OPD of the Institute of Obstetrics and Gynaecology, Egmore, Chennai. Based on the following inclusion and exclusion criteria females of this group were selected.

Inclusion Criteria

1. Patients with recently confirmed diagnosis of polycystic ovary syndrome based on history and clinical examination of abdomen and pelvis.

2. Ultra sound finding of unilateral/bilateral polycystic ovaries.

3. Normal TSH and prolactin levels indicating normal functioning of thyroid and pituitary.

4. Subject of any BMI.

Exclusion Criteria

1. Patients with confirmed diagnosis of PCOS who were already on treatment with insulin sensitizing medications or gonadotropin releasing hormone (GnRH) agonists like clomiphene citrate.

2. Patients with PCOS who had undergone any form of surgical procedure for infertility.

3. Patients with PCOS and associated diabetes mellitus.

4. Patients with PCOS and associated features of hypothyroidism as evidenced by TSH values.

5. Patients with adrenal or ovarian tumors.

Sample Collection

For all the 88 subjects of the study 6ml of peripheral venous blood was withdrawn under sterile conditions with disposable syringes after overnight fasting. One ml of blood was transferred into the test tube containing a pinch of potassium oxalate and sodium fluoride (3:1 mixture) for plasma glucose estimation. In to the other plain tube without any anticoagulant 5ml of remaining blood was transferred. The seperated serum from this tube was pipetted into a centrifuge tube and was centrifuged at 2500 revolutions per

minute for five minutes, to get clear serum without any cells. 0.5ml of the above serum was transferred into an ependroff and stored at -70^{o} for the estimation of serum insulin. From the remaining serum, triglycerides (TGL) total cholesterol (TC) and high density lipoprotein cholesterol (HDLc) were measured within 6 hours of blood collection by enzymatic methods using commercial kits.

The biochemical parameters undertaken for the study were determined using the following methodologies.

ESTIMATION OF PLASMA GLUCOSE

Method : Glucose oxidase peroxidase (GOD/POD)

Kit used : Autopak of Bayer Diagnostics

Principle

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconicacid} + H_2O_2$$

$$H_2O_2 + \text{Phenolic compound} + \text{4 aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{pink coloured compound (Quinonemine)} + 2H_2O$$

The intensity of pink coloured compound is proportional to glucose concentration and was measured at 505nm.

Reagents

1. Glucose reagent - consist of Glucose oxidase, peroxidase, 4-Aminoantipyrine , 4- hydroxy benzoic acid and phosphate buffer.

2. Glucose standard - 100mg / dl

Sample : Clear plasma

Reagent Reconstitution

Working solution was prepared by dissolving one tablet of glucose reagent in 20ml of deionised water with continuous stirring. It was stored in brown bottle.

Procedure

Reconstituted reagent was brought to room temperature prior to use.

Assay Parameters

Reaction Type	:	End Point
Reaction slope	:	Increasing
Wavelength	:	505nm (490 - 530)
Incubation time	:	15 min at 37^{o}C
Sample volume	:	10µl
Reagent volume	:	1.0 ml
Zero setting	:	Reagent blank.

To one ml of working solution 10µl of plasma was added and incubated at 37^{o}c for 15 minutes and absorbance was measured at 505nm.

Reference range

Fasting plasma glucose - 70-100 mg/dl.

ESTIMATION OF INSULIN

Method : Insulin was estimated by ELISA method using active Insulin ELISA kit of Diagnostic System Laboratories.

Principle

This is an "One - step" sandwich type enzyme immunoassay in which the antigen is sandwiched between two antibodies. The assay standards / controls / and unkown serum samples were incubated with a horseradish peroxidase (HRP) - labelled anti insulin antibody in microtitration wells which had been coated with another anti - insulin antibody so that antibody - antigen antibody complex was formed. After incubation and washing of excess, the wells were incubated with the substrate, tetramethyl benzidine (TMB). An acidic stop solution is then added and the degree of enzymatic turnover of the substrate was determined by dual wave length absorbance at 450 and 620 nm.

Reagents

1. Anti - insulin - coated micro titration-strip

   One strip holder contains 96 polystyrene microtitre wells with anti - insulin antibody immobilized to the inside wall of each well.

2. Insulin standards : (Lyophilized).

   Vials containing different concentration of standards like 0, 3.0, 10.0, 50.0, 100; 300 µu/ml. Insulin in human serum with a non - mercury perservative. Standards were reconstituted with 0.5 ml deionized water.

3. Insulin controls : (Lyophilized)

   2vials containing low and high concetration of insulin in human serum with a non - mercury preservative. Reconstitution of each vial done with 0.5 ml of deionized water.

4. Insulin antibody - enzyme conjugate concentrate :

   One vial containing anti - insulin antibody conjugated to horse radish peroxidase in buffer with a non - mercury preservative.

5. Assay buffer:

   Contains protein based buffer.

6. TMB chromogen solution :

   One vial containing a solution of tetramethylbenzidine (TMB) in citrate buffer with hydrogen peroxide.

7. Wash concentrate :

   Contains buffered saline with a non ionic detergent.

8. Stopping solution :

   0.2 M sulfuric acid.

Specimen : Clear fasting serum.

Procedure

All specimens and reagents of the kit are brought to room temperature (25°C). The reagents and samples were thoroughly mixed before use by gentle inversion. 25 µl of standards / controls and samples were pipetted into the appropriate wells. 100 µl of antibody enzyme conjugate solution was added into each well and was incubated in a rotar shaker at fast speed (500 - 700 rpm). Then each well was aspirated and washed 5 times with wash solution. Then the plates were dried by blotting. 100 µl of TMB chromogenic substrate was added into each well and incubated at fast speed in rotar shaker at (500 - 700) rpm for 10 minutes at room temperature. Then 100 µl of stop solution was added to each well. The absorbance was read immediately at 450 nm. The absorbance of standards / controls are plotted with concentration in X axis and optical density in Y axis. With the standard curve the concentration of samples were found out.

Reference range

Fasting insulin (Adult) 2.1 - 30.8 µu/ml.

ESTIMATION OF SERUM TRIGLYCERIDE

Method

Enzymatic colorimetric method

Kit used

Autopak of Bayer Diagnostics

Principle

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{Fatty Acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone Phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{ADPS} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + 2H_2O$$

GPO = Glycerol - 3- Phosphate Oxidase

ADPS = N-Ethyl-N-Sulfopropyl-n-anisidine

The intensity of purple coloured complex formed during the reaction is directly proportional to the triglycerides concentration in the sample and is measured at 546 nm.

Reagents

Reagent 1 (Enzymes / Chromogen) :

Lipoprotein lipase	≥ 1100 U/L
Glycerol kinase	≥ 800 U/L
Glycerol-3-Phosphate Oxidase	≥ 5000 U/L
Peroxidase	≥ 350 U/L
4-Aminoantipyrine	0.7 mmol/L
ATP	0.3 mmol/L

Reagent 1A (Buffer) :

Pipes buffer, pH 7.50	50 mmol/L
ADPS	1 mmol / L
Magnesium salt	15 mmol/L

Standard (Triglycerides 200 mg/dL):

Glycerol (Trig. equivalent)	2 g/L

Reagent Reconstitution

Reagents were brought to room temperature. Contents of one bottle of reagent 1 was dissolved with one bottle of reagent 1A. It was mixed gently by swirling.

Procedure

The samples and the reconstituted reagent were brought to room temperature prior to use. The following general system parameters were used to with this kit :

General System Parameters

Reaction Type	:	Endpoint
Reaction Slope	:	Increasing
Wavelength	:	546 nm (520 - 570)
Flowcell temp.	:	30°C
Incubation	:	5 min at 37°C
Sample vol.	:	10 µl
Reagent vol.	:	1.0 ml
Std. concentration	:	200 mg/dl
Zero setting with	:	Reagent Blank

In the test tube following was dispensed

	Blank	**Standard**	**Test**
Reconstituted Reagent	1 ml	1 ml	1 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

Reading was taken after 5 minutes incubation at 37°C.

Reference Values

Males	:	60 - 165 mg/dl
Females	:	40 - 140 mg/dl

ESTIMATION OF SERUM TOTAL CHOLESTEROL

Method

Cholesterol Esterease - Cholesterol Oxidase

Kit used

Autospan of Span Diagnostics Ltd

Principle

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + 4\text{-}H_2O$$

The concentration of cholesterol in the sample is directly proportional to the intensity of the red complex (Red Quinone) which is measured at 500 nm.

Reagents

Reagent 1 (Enzymes / Chromogen)

Cholesterol esterase	-	≥ 200 U/L
Cholesterol oxidase	-	≥ 250 U/L
Peroxidase	-	≥ 1000 U/L
4-Aminoantipyrine	-	0.5 mmol/L

Reagent 1A (Buffer)

Pipes buffer, pH 6.90	-	50 mmol/L
Phenol	-	25 mmol/L
Sodium Cholate	-	0.5 mmol/L

Standard (Cholesterol 200 mg/dL)

Cholesterol	-	2 g/L

Reagent Reconstitution

Reagents were brought to the room temperature contents of one bottle of reagent 1 was dissolved with one bottle of reagent 1A. It was mixed by gentle swirling.

Procedure

The samples and the reconstituted reagent were brought to room temperature prior to use.

Instrument was set using following system parameters

General System Parameters

Reaction type	:	Endpoint
Reaction slope	:	Increasing
Wavelength	:	500 nm (492 - 550)
Flowcell temp.	:	30°C
Incubation	:	5 min at 37°C
Sample vol.	:	10 µl

Reagent vol. : 1.0 ml

Std. concentration : 200 mg/dl

Zero setting with : Reagent Blank

In the tube following was dispensed :

	Blank	**Standard**	**Test**
Reconstituted Reagent	1 ml	1 ml	1 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

Reading was taken after 5 minutes incubation at 37°C.

Reference Values

Cholesterol: Serum / Plasma: 150 - 260 mg/dl

Normal cholesterol level is affected by stress, age, pregnancy and hormonal balance.

ESTIMATION OF HDL - CHOLESTEROL

Method

Posphotungstate method

Kit used

Autopak of Bayer Diagnostics

Principle

Chylomicrons, VLDL (Very low density lipopproteins) and LDL fractions in serum or plasma are separated from HDL by precipitating with phosphotungstic acid and magnesium chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant is assayed with enzymatic cholesterol method, using cholesterol esterase, cholesterol oxidase, peroxidase and the chromogen 4-aminoantipyrine / Phenol.

Reagents

Reagent 1 (Enzymes / Chromogen) :

Cholesterol esterase	$\geq$ 200 U/L
Cholesterol oxidase	$\geq$ 250 U/L
Peroxidase	$\geq$ 1000 U/L
4-Aminoantipyrine	0.5 mmol/L

Reagent 1A (Buffer) :

Pipes buffer, pH 6.90	50 mmol/L
Phenol	24 mmol / L
Sodium Cholate	0.5 mmol/L

Reagent 2 (Precipitating Reagent) :

Phosphotungstic Acid	2.4 mmol/L
Magnesium Chloride	39 mmol/L

Standard (HDL Cholesterol 50 mg/dL) :

Cholesterol	0.5 g/L

Reagent Reconstitution

Reagents were brought to room temperature. Contents of one bottle of reagent 1 was dissolved with one bottle of reagent 1A. It was mixed gently by swirling.

Procedure

1. Precipitation

Following was dispensed into centrifuge Tubes :

	Test
Sample	0.20 ml (200 µl)
Precipitating Reagent 2	0.20 ml (200 µl)

Mixed Well

It was centrifuged at 1500 g or 3500 - 4000 rpm for 10 minutes.

Clear supernatant was immediately separated and cholesterol content was determined as follows :

II CHOLESTEROL ASSAY

The following general system parameters was used with this kit :

General System Parameters

Reaction type : Endpoint

Reaction slope : Increasing

Wavelength : 500 nm (492 - 550 nm)

Flowcell temp. : 30°C

Incubation : 5 min at 37°C

Sample vol. : 20 µl

Reagent vol. : 1.0 ml

Std. concentration : 100 mg/dl

Zero setting with : Reagent blank

In the test tube following was dispensed

	Blank	**Standard**	**Test**
Reconstituted Reagent	1 ml	1 ml	1 ml
Standard	-	20 µl	-
Supernatant	-	-	20 µl

Reading was taken after 5 minutes of incubation at 37°C.

Reference Values

Serum / Plasma HDLc : 30 - 70 mg/dl

VLDL AND LDL CHOLESTEROL

These parameters were calculated using Friedwald's formula which is given below.

LDL c = TC - (HDL c + VLDL c)

VLDLc = TGL / 5

Glucose insulin ratio is arrived directly from Glucose and Insulin levels from the formula G/I.

HOMA - IR - This is arrived at using the following formula.

$$\text{HOMA - IR} = \frac{[(\text{Glucose in mg / dl} \times 0.05551) \times \text{Insulin in } \mu\text{u/ml})]}{22.5}.$$

RESULTS

The estimated levels of fasting serum insulin and other biochemical parameters namely fasting plasma glucose, serum triglycerides, total cholesterol, high density lipoprotein cholesterol and the calculated parameters, very low density lipoprotein cholesterol of all the subjects selected for study irrespective of the grouping are tabulated in master Table No.I, along with the calculated Glucose Insulin ratio and Homeostasis model assessment insulin resistance (HOMA - IR) which are the indices of Insulin resistance. Of the selected subjects serial No.1-30 in the above table consists of the control subjects and serial No.31-88 consists of patients with PCOS.

Table No.II gives the levels of blood parameters in apparently normal women (i.e serial. No.1-30 of the master table I) who form the control group while table No.III represents the same in women with PCOS (i.e serial No.31-88 of master table I). The mean level of each parameter along with its standard deviation (S.D.) is also shown in the above 2 tables namely table II and III. The mean levels of each blood parameter in the two groups are also shown as bar diagram in fig.No.6 to 8.

To determine how far the levels of blood parameters varied in the 2 groups the mean levels in table III are compared with that of table II in table IV. The statistical significance for the variation in the levels of the blood parameters between the 2 groups is determined from the p value which is

arrived at using the student's 't' test.

The correlation of plasma insulin to fasting plasmaglucose (FPG) and the lipid parameters are shown in table V. The correlation have been arrived at using Karl Pearson's correlation coefficient. The above correlation is also depicted in scatter diagrams from Fig.No.9 to 16.

DISCUSSION

The validity of the mean values obtained for the analysed blood parameters in apparently normal women to be used as reference range for the study is first scrutinised.

The mean value of 82.37 ± 7.09 mg/dl obtained for fasting plasma glucose is well within the reference range of 70 - 100 mg / dl of the kit methodology adopted for its determination. Moreover the value is well within that quoted in standard text books for fasting plasma glucose[82].

The value of 24.87 ± 16.38 µu/ml obtained for fasting insulin is found to be at the higher end of 2 - 25 µu/ml quoted by carl. A Burits[82]. The higher reference range obtained can probably be attributed to the fact that obese individuals have not been excluded in the control group; it is a well known fact that obesity is associated with insulin resistance which can lead to hyperinsulinemia[38,42]. Moreover it can also be due to the different race of the subjects analysed in this study compared to carl. A Burtis who arrived at from western population. However it coincides much better with the reference range of 2.1 to 30.8 given in the kit methodology adopted for its analysis Similarly the mean levels of the lipid parameters are well within the reference range of their respective methodologies and that of standard text books[82]. Hence the acceptance of the mean levels of the analysed biochemical parameters namely 82.37 ± 7.09 for fasting plasma glucose, 24.87 ± 16.38 for fasting serum

insulin, 103.70 ± 29.31 for serum TGL, 164.27 ± 26.99 for total cholesterol, 46.71 ± 9.44 for HDL c obtained from apparently normal women as the reference range for the study is valid. Therefore levels of the calculated parameters obtained from the above values namely 23.11 ± 14.34 for VLDL c, 93.02 ± 26.43 for LDL c, 6.13 ± 5.96 for glucose insulin ratio and 5.09 ± 3.43 for the HOMA - IR are acceptable.

On scrutinising Table IV where the mean levels of the biochemical parameters are compared with the reference range obtained in the study it is found that among the analysed parameters insulin, TGL and total cholesterol have increased from that of the level in controls to highly significant levels (P = .003 for insulin, .001 for TGL and TC). On the other hand HDL c is found to be significantly lowered (p = .02). Among the calculated parameters HOMA - IR, VLDL c and LDL c are increased to highly significant levels (p = .003 for HOMA - IR, .004 for VLDL C and .001 for LDL c), while the G/I is decreased significantly (p - .04).

Fasting plasma insulin which shows a highly significant elevation in PCOS from the control level is attributed to the insulin resistance as it has been well established that there is a strong association between PCOS and insulin resistance which will lead to impaired glucose tolerance and compensatory hyperinsulinemia which is the most significant metabolic abnormality in PCOS[46,47,84-88]. In the study the plasma insulin the most significant abnormality of PCOS has increased to highly significant levels but plasma glucose does not

show any statistical elevation contrary to the finding of Dunaiff A.

The state of insulin resistance in PCOS as reviewed though not well understood, can be due to :

1. Peripheral target tissue resistance to insulin which can be the result of auto antibodies to insulin receptors leading to decrease in insulin binding; post receptor defects such as abnormal pattern of phosphorylation of serine and tyrosine residues of insulin receptors and a decrease in insulin receptor sites in target tissues.

2. Decreased hepatic clearance of insulin due to decreased hepatic extraction.

Careful scrutiny of the levels of insulin in the 58 PCOS subjects revealed that 42 of them have mild to moderate elevation as per the criteria of C.Ronal Kaln et al., the remaining 16 had levels within reference range. This coincides with the finding of catherin Marin Deugarte et al., who have stated that though insulin resistance is a common abnormality in PCOS it is not an universal feature[78].

The abscence of any significance for glucose in PCOS inspite of insulin resistance is attributed to the prevention of plasma glucose elevation by the compensatory hyper insulinemia which is a consequence of insulin resistance. This fact has been also emphasised by Serdar E. Bulun et al., who have stated

that in PCOS subjects with insulin resistance normal glucose level is maintained at the expense of increased circulating insulin to overcome the underlying defect[59].

Assessment of the 2 parameters arrived at from plasma glucose and insulin levels reveal the following. The ratio derived from the above plasma glucose and insulin is found to be significantly reduced in PCOS ($p = .04$). This is only natural because out of the 2 parameters involved in the calculation of the ratio the numerator glucose level has not changed statistically while the denominator insulin level has increased to highly significant levels in PCOS.

HOMA - IR is increased to highly significant level in PCOS ($p .003$) because it is arrived at using the formula.

$$\text{HOMA - IR} = \frac{\text{glucose in mg/dl x 0.05551 x insulin in µu/ml}}{22.5}$$

Even though plasma glucose level does not show any significant elevation insulin is increased to highly significant levels which increases the HOMA - IR to a highly significant levels in PCOS.

As the above 2 parameters namely glucose insulin ratio and HOMA - IR indicate insulin resistance careful scrutiny of their levels indicate the following. The levels of glucose insulin ratio when analysed keeping in mind the criteria of Marilyn R. Richard[15] that levels of glucose insulin ratio of more than 4.5

excludes insulin resistance and less than that indicate insulin resistance it is observed that 46 PCOS subjects in this study have values less than 4.5 while the remaining 12 have values higher to it. Hence as per the above values 79.3% of PCOS subjects have insulin resistance in this study which is higher to the percentage arrived by carmina E et al., which is 65.4[79]. This difference in the percentage of insulin resistance in PCOS can be attributed to the racial difference of the subjects analysed in this study from that of the former study by carmina E et al., as genetic predisposition has also been identified as one of the etiological causes for the syndrome[48]. Moreover even in controls group where the mean is 6.13, 15 out of 30 subjects have G/I ratio less than 4.5 indicating that 50% of the controls selected have insulin resistance which has led to hyperinsulinemia in these subjects. The lower glucose insulin ratio in some control subjects can be attributed to the obesity of these individuals who have been selected in the control group as it has been reviewed that insulin resistance is associated with obesity[38,39,40].

HOMA - IR levels scrutiny based on the statement of Dr.Elizabeth G. Nabel who had found that HOMA - IR more than 3.8 indicates insulin resistance it is observed that 46 PCOS subjects have HOMA - IR levels more than 3.8 assuring that 79.3% of subjects with PCOS have insulin resistance which is similar finding to that obtained from G/I ratio. But when mean of HOMA - IR in controls of the study is observed it is found that it is 5.09 ± 3.34 which is also greater to the above cut off level of 3.8. Therefore it is inferred

that certain subjects in the control group of this study have also insulin resistance which leads to hyperinsulinemia and therefore has raised the reference range of insulin in the control group higher to that quoted in the standard text books[82]. In this study fifteen subjects of the control group have HOMA - IR greater than 3.8 indicating as in glucose insulin ratio that 50% of the control selected have insulin resistance which correlates with obese subjects.

Among the lipid parameters, TGL, TC, VLDLc and LDLc show a highly significant elevation from the level in controls, while HDLc shows a significant decrease. It is well documented fact that insulin resistance in PCOS can cause alteration in lipid metabolism[59]. Hence the elevation of lipid parameters can be the result of dyslipidemia which in one of the complication in PCOS.

The various causes pertaining to the increased levels of the above lipid parameters are enumerated below.

1. Hyperinsulinemia is known to enhance hepatic very low density lipoprotein synthesis and thus can directly contribute to increased plasma TGL.

2. As the insulin resistance restricts the action of lipoprotein lipase more of adipose tissue TGL is hydrolysed leading to increase in free fatty acids which inturn will elevate TGL synthesis $\rightarrow$ VLDL production $\rightarrow$ LDL production.

The significant decrease in HDL-c in PCOS can be attributed to the increased rate of HDL degradation which can exceed the rate of its synthesis.

When the plasma insulin levels are correlated with fasting plasma glucose and lipid parameters the following are revealed from the table No.V and is also in evident in scatter diagram in Fig.No.9 to 16.

1. Fasting plasma insulin with fasting plasma glucose shows poor correlation in both control as well as the PCO's group.

2. Fasting plasma insulin with TGL and TC shows in poor correlation in controls and a fair positive correlation in PCOS.

3. Fasting plasma insulin with HDL-c shows a poor correlation in controls and a fair correlation in PCOS.

CONCLUSION

From the discussion held sofar on the results obtained in the study on PCOS the following conclusion is arrived at regarding the biochemical parameters.

1. The plasma insulin in control in 24.87 ± 16.38 µu/ml which is the reference range for the study.

2. Plasma insulin is elevated in PCOS above the reference range indicating hyper insulinemia in this group.

3. Glucose insulin ratio and HOMA - IR the marker of insulin resistance are decreased and increased from the reference range respectively.

4. Analysis of glucose insulin ratio and HOMA reveals that 79.3% of PCOS are insulin resistant.

5. Correlation of insulin with plasma glucose is poor in both control and PCOS. Correlation of insulin with TGL and TC and HDLc are poor in control but has fair positive correlation with TGL and TC and fair negative correlation with HDLc in PCOS.

6. 50% of subjects in the control group have insulin resistance.

SCOPE FOR FURTHER STUDY

1. Insulin resistance can be studied in obese and non obese controls and PCOS.

2. Insulin resistance can be studied in other family members of these patients.

3. Study of levels of insulin and glucose simultaneously by oral glucose tolerance test in PCOS.

4. Insulin resistance can be analysed along with sex hormones.

5. Levels of leptin, adiponectin can be studied in PCOS patients.

6. Study of levels of plasminogen activator inhibitor in these patients.

BIBLIOGRAPHY

1. Ruijnwu and Marcin T.Stankiewicz Medical Journal of Australia 2004 180(3): 132 - 137.

2. Norman RJ Hyperandrogenemia and the ovary. Mol Cell Endocrinol 2002; 191: 113 - 119.

3. Lobo RA, Carmina E. The importance of diagnosing the polycystic ovary spndrome. Ann Intern Med 2000; 132 : 989 - 993.

4. Farquhar CM, Birdsall M, Manning P. et al. The prevalence of polycystic ovaries on ultra sound scanning in a population of randomly selected women. Aust NZJ Obsted Gynacol 1994; 34: 67 - 72.

5. Polson DW Adams J, Wadsworth J, et all. polycystic ovaries a common finding in normal women Lancet 1998; 1: 870 - 872.

6. Guzick D. Polycystic ovary syndrome : Symptamatology, patho physiology, and epidemiology. Am J obstet Gynecol 1998; 179 : 887 - 8.

7. Nestler JE, Strauss JF 3d Insulin as an effector of human ovarian and adrenal steroid metabolism. Endocrinol Metab Clin North Am 1991; 20 : 807 - 23.

8. Nestler JE Role of obesity and insulin in the development of anovulation. In : Filicoli M, Flamigri C, eds. Ovulation induction : basic science and clinical advances : procedings of the symposium on ovulation Induction : Basic Science and clinical advance, 20 - 22 January 1994, Palm Beach, Florida. USA. New York : Excerpta Media, 1994 : 103 - 14.

9. Jeffcoates - Principles and Gynaecology 546 - 547.

10. Agalwal SK, Judd HL, Magoffin DA, A mechanism for the suppression of estrogen production in PCOS. Journal Clin. Endocrinol Metah 81 : 3686, 1996.

11. Casper RF, Greenblatt EM, Laparopscopic ovarian cautery for induction of ovulation in women with polycystic ovarian disease, seminass reprod. Endocrinol 8 : 2080, 1990.

12. Leon speroff M.D Clinical Gynaecologic Endocionology and infertility 7th Edition - 474, 479.

13. Ovarian polycystic disease : Article by Nadia K. Waheed M.D. Rawalpind Medical College, Pakistan.

14. Marilyn R. Richardson, M.D, current perspectives in polycystic ovary syndrome. Journal of the American Academy Family Physicians. Vol.68 Mo.4 (Aug 15, 2003).

15. Melissa H, Hunter, M.D., and James J et al., Polycystic ovary syndrome : It's not just fertility. Journal of the American Academy Family Physicians Vol. 62 / No.5 (Sep 1, 2000).

16. C.Ronalkahn, Gordon C weir, Robert J Smith et al., Joselin Diabetes 13th Edition - 483 / 285.

17. C.Ronalkahn, Gordon C weir, Robert J Smith et al., Joselin Diabetes - 14th Edition - 156.

18. Gold stein BJ, Kahn Cr. Insulin allergy and insulin resistance. In : Lichtenstein LM, Fouci AS, eds current therapy in allergy, immunology and rheumatology lewiston: BC Decker, 1988 : 327 - 330.

19. Caro JF, Dohm LG, Pories WJ, et al. Cellular alterations in liver, skeletal muscle and adipose tissue responsible for insulin resistance in obesity and type II diabetes. Diabetes, Metal Rev. 1989; 5 : 665 - 689.

20. Cusik, Maezono K, Osman A, et al. Insulin resistance differentially affects the PI3 - kinase - and MAP Kinase Mediated signalling in human muscle. J Clin Invest 2000; 105 : 311 - 320.

21. Accili D. Molecular defects of the insulin receptor gene. Diabetes Metab Rev. 1995; 11 : 47 - 62.

22. Gavin JR III, Roth J, Neville DM Jr. et al. Insulin - department regulation of insulin receptor concentration, Proc Natt Acad Sci. USA 1974; 71 : 84 - 88.

23. Bar SR, Harrison LC, Muggeo M, et al Regulation of insulin receptors in normal and abnormal physiology in humans. Adv Intern Med. 1979; 24:23-52.

24. Kerouz NJ, Horsch D, Pons S, et al. Differential regulation of insulin receptor substrates 1 and 2 (IRS and IRS - 2) and phosphatidyl ionositol - 3 kinase

isoforms in liver and muscle of the obese diabetic . Journal and Clinical Invest 1997; 100 : 3164 - 3.

25. Turnbow Ma, Smith LK, Garner CW. The oxazolidinedione CP - 92, 768 - 2 partially protects insulin receptor substrate - 1 from dexamethasone down regulation in 3T3 LI adipocytes. Endocrinology 1995; 136 : 1450 - 1458.

26. Reei, Fisha TL, Thomas J, et al. Regulation of insulin / insulin - like growth factor - 1 signaling by proteosome mediated degradation of insulin receptor substrate 2 Journal Biol chem 2001; 276: 40362 - 40367.

27. Zhande R, Mitchell JJ, Wuj, et al. Molecular mechanism of insulin-induced degradation of insulin receptor substrate 1 Mol. Cell Niol 2002; 22:1016-1026.

28. Mothe, I, Van Obberghan E, Phosphorylation of insulin receptor substrate - 1 on multiple serine residues, 612, 662 and 731 modulates insulin action. J Biol Chem 1996; 271 : 11222 - 11227.

29. Gaoz, Hwang D, Bataille F. et al Serine phosphorylation of insulin receptor substrate 1 (IRS-1) by inhibitor kappa B Kinase (IK) complex. Journal Bio chem 2002; 277 : 48115 - 48121.

30. Emanuelli B, Peraldi P, Filloux c, et al. SOCS - 3 inhibits insulin signaling and is up-regulated in response to tumour necrosis factor - alpha in the adipose tissue of obese mice. Journal Biol Chem 2001; 276; 47944 - 47949.

31. Emanuelli B, Peraldi P, Fillouxe et al. SOCS - 3 is an insulin - induced negative regulator of insulin signalling Journal Biol Chem 2000; 275 : 15985 - 15991.

32. Ruil, Yuan M, foranty D, et al. SOCS - 1 and SOCS - 3 block insulin signaling by ubiquitin - mediated degradation of 1RS2. Journal Biol Chem 2002; 277 42394 - 42398.

33. O' Rahilly S, Farroqi IS, Yeo GS, etal Minireview: human obesity - lesson from monogenic disorders (review), Endocrinology 2003; 144 : 3757 - 3764.

34. Torlay D. Bellus GA, Munro CS. Genes, growth factors and acanthosis nigricans. Br. J. Dermatol 2002; 147 : 1096 - 1101.

35. Taylor SI. Insulin action, insulin resistance, and type 2 diabetes mellitus. In : Seriver CR, Baudette AL, Sly ws, et al eds. The metabolic and molecular bases of inherited diseases, 8th ed New York; Mc Graw - Hill, 2000 : 1433 - 1470.

36. Virkamaki A, Ueki K, Khan Cr. Protein protein interaction in insulin signaling and the molecular mechanism of insulin resistance. Journal Clin Invest 1999; 103 : 931 - 943.

37. Krook A. Brueton L, P' Rahilly s. Homozygous nonsense mutation in the insulin receptor gene in infant with leprechaunism. Lancet 1993; 342 : 277 - 278.

38. Reitman ML, Ariogly, E, Gavrilova O, et al - Lipoatrophy revisited. Trends endocrinol Metab 2000; 11 : 410 - 416.

39. Comuzzie AG, Allison DB. The search for human obesity genes. Science 1998; 280 : 1374 - 1337.

40. Hill J.O., Peters JC. Environmental Contributions to the Obesity epidemic. Sciences 1998; 280 : 1371 - 1374.

41. Hotamisligil GS, Peraldi, P, Budrari A, et al., ISR - 1 mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α and Obesity induced muslces resistance. Science 1996; 271 : 665 - 668.

42. Hawsen I, Tsalikian E, Beawyere B, et al., Insulin resistance in acromegaly: defects in both hepatic and extrahepatic insulin. Am. Journal. Physiol, 1986; 250 - (3 Pt 1) : E269 - E273.

43. Cefalu WT, Wang ZQ, Werled S, et al., contribution of viseral fat mass to the insulin resistance of aging. Metabolism 1995; 44 : 954 - 959.

44. Peterson KF, Brefroy D, Defour S. et al., Mitochondial dysfunction in the elderly : Possible role in insulin resistance. Science 2003 : 300 : 1140 - 1142.

45. Ardawi MS, Rouzi AA, Plasma adiponectin and insulin resistance in women with polycystic ovary sundrome. Journal Fertil Steril 2005. June ; 83 (6) : 1708-16.

46. Burghen GA et al. Correlation of hyper androgenism with hyper insulinism in polycystic ovariandisease. J . Clin Endocrinol Metab 1980 : 50 : 113 - 6.

47. Dunaif A. Insulin resistance and the polycystic ovary syndrome : mechanism and implicantions for pathogenesis. Endocr Rev. 1997; 18 : 774 - 800.

48. Sozen I, Arici A, Hyper insulinism and its interaction with hyper androgenism in polycystic ovary sundrome. J. Obstet Gynecol Sur. 2000 May; 55(5):321-8.

49. Ciaraldi TM El - Roeiy A, Madar Z, Reichart D, Olefsky JM. Yen SCC.

Cellular mechanism of insulin resistance in polycystic ovarian syndrome. J. Clin. Endocrinol Metah. 1992; 75 : 577 - 83.

50. Marsden PJ, Murdoch A, Taylor R. Severe impairment of insulin action in adipocytes from amenorrheic subjects with polycystic ovary syndrome. Metah. Clin. Exp. 1994; 43 : 1536 - 42.

51. Dunaif A, Xia J, Book C-B Schenker E, Tang Z. Excessive insulin receptor phosphorylation in cultured fibroblasts and in skeletal muscle : a potential mechanism for insulin resistance in the polycystic ovary syndrome. Journal Clin. Invest. 1995; 96 : 801 - 10.

52. Barhieri RL, Makris A, Randall RW, *et al.,* : Insulin stimulates androgen accumulation in incubation of ovarian stroma obtained from women with hyperandrogenism. Journal Clin. Endocrins. Metah, 1986; 62 : 904 - 10.

53. Nestler. JE, Jakubowic DJ, de Vargas AF, et al., : Insulin stimulates testosterone biosynthesis by human there cells from women with polycystic ovary syndrome by activating its our receptor and using inositol - glycan mediators as the signal transduction System. Journal Clin. Endocrinol, Metab. 1998; 83 : 2001 - 5.

54. Velazquez EM, Mendoza S, Hamer T, *et al.,* : Metformin therapy in polycystic ovary syndrome reduced hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism 1994; 43 : 647 - 54.

55. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. *et al.,* : Effects of metformin on spontaneous and clomiphene induced ovulation in the polycystic ovary sydrome. New England Journal of Med. 1998; 338 : 1876 - 80.

56. Moghetti P, Castello R, Negri C, et al., : Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivsity in PCOS : Journal Clin. Endocrinol, Metah, 2000; 85 : 139 - 46.

57. Aziz R, Ehrmann D, Legrop R.S. et al., : Troglitazone improves ovulation and hirsulism in the PCOS. J. Clin. Endocrinol Metab. 2001; 86 : 1626 - 32.

58. Venkatesan A, Dunaif A, Corbould A. Insulin resistance in PCOS : progress and paradoxes. Recent Prog. Horm. Res. 2001; 56 : 295 - 308.

59. Williams Text book of endocrinology 10th Edition, pg.633.

60. Toprak S, Yonem A, Cakir B et al., Insulin resistance in non obese patient with PCOS. Horm. res. 2001; 55(2) : 63 - 70. Related articles.

61. Bernard L, Christin - Maitres, Basille C, et al., Insulin resistanced and polycystic ovary syndrome. Gynecol. Obstet. Fertil. 2003 Feb; 31(2) : 109 -16, Related articles.

62. Goodarzi, M.O, Erickson S, Port SC, et al., Relative impact of insulin resistance and obesity on cardio vascular risk factors in polycystic ovary syndrome. Metabolism, 2003 Jun; 52(6) : 713 - 9, Related articles.

63. Dunail A, Segal KR, Shelly DR, et al., : Evidence for distinctive and intrinsic defect in insulin action in PCOS. Diabetes 1992; 41 : 1257 - 1266.

64. Nestler JE, Powers LP, Matt DW, et al., : A direct effect of hyper insulinemia on serum sex hormone- binding, globulin levels in obese women with polycystic ovary syndrome. J. Clin. Endocrinol Metab. 1991; 72 : 83 - 89.

65. Moghetti P, Castello R, Negric et al., : J. Clin. Endocrinol, Metab, 1996; 81 : 881 - 886 / 85 : 139 - 146.

66. Elkind HIrsh KE, Valdes CT, McConnel TG, et al., : Androgen responses to acutely increased endogenous insulin levels in hyperandrogenic and normal cycling women. Fertil Steril, 55 : 486, 1991.

67. Smith S, Ravnikar VA, Barbieri RL, Androgen and insulin response to an oral glucose challenge in hypernadrogenic women, Fertil. Steril, 48 : 72, 1987.

68. Kiddy DS, Hamilton - Fairely D, Seppala M, et al., : Diet - induced changes in sex hormone binding globulin and free testosterone in women with normal or polycystic ovaries : correlation with serum insulin and insulin - like growth factor - I. Clin. Endocrinol, 31 : 757, 1989.

69. Barbieri RL, Malcris A, Rejan KJ. Insulin stimulates androgen accumulation in incubations of human ovarian stroma and theca. Obstet. Gynecol. 64 : 735, 1984.

70. Barbieri RL, Makris A, Randall RW, et al., : Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. Journal. Clin. Endocrinol. Metab, 62 L 904, 1986.

71. Nestler JC, Barlascini CO, Malt DW, et al., Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic. Ovary syndrome. Journal. Clin. Endocrinol, Metab. 68 : 1027, 1989.

72. Dunaif A, Scoh D, Finegood D, Quintane, et al., : The insulin sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. Journal Clincal. Endocrinol, Metab. 81 : 3292, 1996.

73. Ehrmann DA, Cavaghan MK, Imperial J. Sturis J , et al., : Effects of

metformin on insulin secretion, insulin action and ovarian steroidogensis in women with polycystic ovary syndrome, J. Clini. Endocrinol. Metab, 82 : 524, 1997.

74. Dunaif A, Green G, Futterweit W, Dobrjansky A. Suppression, of hyperandrogenism does not improve peripheral or hepatic insulin resistance in the polycystic ovary syndrome. J. Clin. Endocrinol, Metab. 70 : 699, 1990.

75. Dale PO, Tanbo T, Djoseland O, Jervell J et al., Persistence of hyperinsulinemia in polycystic ovary syndrome after ovarian suppression by gonadotropin releasing hormone agonist, Acta Endocrinol 126 : 132, 1992.

76. Femieux S., Lewis GF, Ben - Chetrit A, *et al.,* : Correlation of hyperandrogenemia by laproscopic ovarian cautery in women with polycystic ovarian syndrome is not accompanied by improved insulin sensitivity or lipid - lipoprotein levels, J. Clin. Endocrinol Meta, 84 : 4278, 1999.

77. Tarkun I, Cetinarslan B *et al.,* : Effect of rosiglitazone on insulin resistance, C-reactive protein and endothelial function in non - obese young women with polycystic ovary syndrome. Eur Journal Endocrinol, 2005, Jul; 115 - 21.

78. DeUgarte CM, Bartolucci AA, Azziz R. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertil Steril. 2005, May; 83(5) : 1453 - 60.

79. Carmina E, Lobo RA. Use of Fasting blood to assess the prevalence of insulin resistance in women with polycystic ovary syndrome. Fertile Steril. 2004, Sep; 82(3) : 6661 - 5.

80. Legro RS, Castracane VD, Kauffman RP. Detecting insulin resistance in polycystic ovary syndrome : Purposes and pitfalls. Obstet. Gynecol. Surv. 2004, Feb; 59(2) : 141 - 54.

81. Archard C, Thiers J, Le virilisme pilaire et son associaton a 1' insuffiance glycolytique (diabets des femmes a barbe) Bll Acad Natl Med 86 : 51, 1921.

82. Carl A, Burtis, Edward R. Ashwood, David E. Burns, Tietz text book of Clinical Chemistry and molecular diagnostics 3rd Edition.

83. Chang RJ, et al. Insulin resistance in nonobese patients with polycystic ovarian disease. J.Clin Endocrional Metab 1983; 57 : 356 - 9.

84. Dunaif A et al. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 1989; 38 : 1165 - 74.

85. Ehrmann et al. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes care 1999; 22 : 141 - 6.

86. Legro RS, et al Prevalence and pridictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome : a prospective,

controled study in 254 affected women. J.Clin Endocrinol Metab 1992; 75 : 557 - 83.

ABBREVIATIONS

PCOS	-	Polycystic ovary syndrome
LH	-	Luteinizing hormone
FSH	-	Follicular stimulating hormone
USG	-	Ultrasonography
TSH	-	Thyroid stimulating hormone
DHEAS	-	Dehydroepiandrosterone sulfate
FFA	-	Free fatty acids
TNFα	-	Tumor necrosis factor
IRS-I	-	Insulin receptor substrate - I
GTT	-	Glucose tolerance test
GI ratio	-	Glucose insulin ratio
HOMA - IR	-	Homeostatic model assessment insulin resistance
TGL	-	Triglycerids
HDLc	-	High density lipoprotein - cholesterol
GnRH	-	Gonadotropin releasing hormone
QUICKI	-	Quantitative insulin sensitivity check index
BMI	-	Body mass index
ELISA	-	Enzyme linked immunosorbent assay
HRP	-	Horse radish peroxidase
TMB	-	Tetramethyl benzidine
FPG	-	Fasting plasma glucose
LDLc	-	Low density lipo protein
VLDLc	-	Very low density lipo protein